Identification of gene-phenotype connectivity associated with flavanone naringenin by functional network analysis

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ABSTRACT

Naringenin, extracted from grapefruits and citrus fruits, is a bioactive flavonoid with antioxidative, anti-inflammatory, antifibrogenic, and anticancer properties. In the past two decades, the growth of publications of naringenin in PubMed suggests that naringenin is quickly gaining interest. However, systematically regarding its biological functions connected to its direct and indirect target proteins remains difficult but necessary. Herein, we employed a set of bioinformatic platforms to integrate and dissect available published data of naringenin. Analysis based on DrugBank and the Search Tool for the Retrieval of Interacting Genes/Proteins revealed seven direct protein targets and 102 indirect protein targets. The protein–protein interaction (PPI) network of total 109 naringenin-mediated proteins was next visualized using Cytoscape. What's more, all naringenin-mediated proteins were subject to Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis by the Database for Annotation, Visualization and Integrated Discovery, which resulted in three ESR1-related signaling pathways and prostate cancer pathway. Refined analysis of PPI network and KEGG pathway identified four genes (*ESR1*, *PIK3CA*, *AKT1*, and *MAPK1*). Further genomic analysis of four genes using cBioPortal indicated that naringenin might exert biological effects via ESR1 signaling axis. In general, this work scrutinized naringenin-relevant knowledge and provided an insight into the regulation and mediation of naringenin on prostate cancer.

INTRODUCTION

Naringenin is chemically known as 5,7-dihydroxy-2-(4-hydroxyphenyl) chroman-4-one (Fig. 1) and abundantly present in grapefruits and citrus fruits and some other food items (*Patel, Singh & Patel, 2018*). Since naringenin was identified from extracts of dormant peach flower buds by *Hendershott & Walker (1959)*, growing attention has been paid to naringenin-mediated bioactivities. In the past decades, a great many studies have been reported that naringenin exhibits a wide range of pharmacological activities, including beneficial effects in liver diseases (*Hernández-Aquino & Muriel, 2018*), antioxidative effect (*Zaidun, Thent & Latiff, 2018*), immunomodulating activity (*Maatouk et al., 2016*), and antitumor activity (*Abaza et al., 2015*; *Gumushan Aktas & Akgun, 2018*; *Liao et al., 2014*;
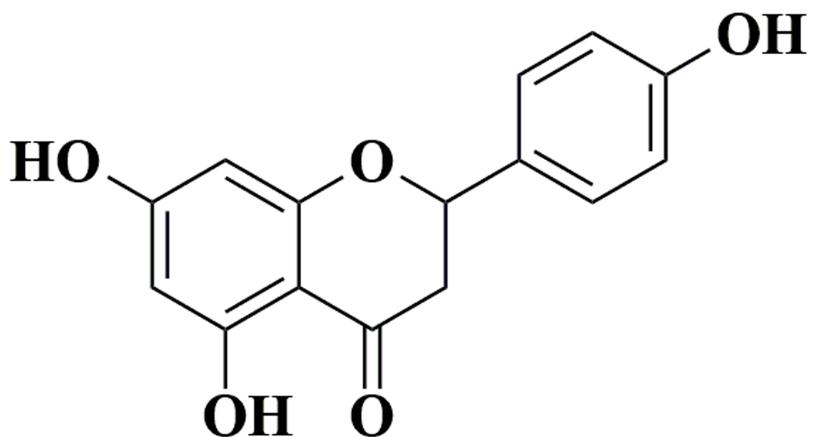
Figure 1 The structure of naringenin. Full-size ☑ DOI: 10.7717/peerj.6611/fig-1

Lim et al., 2017; *Park et al., 2008*; *Sabarinathan, Mahalakshmi & Vanisree, 2011*; *Yen, Liu & Yeh, 2015*). These findings suggest that naringenin appears to be full of therapeutic significance. Hence, it is important to explore the underlying mechanisms of its bioactivities and identify connectivity existing between gene and phenotype liaised by flavanone naringenin in publicly available data. Nevertheless, current knowledge about naringenin function and its mechanisms and targets is based on conventional experiments with high cost and long duration, which are far from integration and comprehension. Accordingly, appropriate and relevant approaches for systematic dissection of available published data are needed to generate authentic and rational lead.

Recently, various types of omics, which encompass genomics, proteomics, and other high-throughput sequencing technology, have been producing and accumulating massive data. Diverse computational approaches have been developed to mine and integrate these data in public databases, which provide researchers opportunities to conduct biomedical research, knowledge discovery, and innovative application (*Lan et al., 2018*). As for exploration of the relationship between naringenin-mediated proteins and their relevant phenotype, the network-based approach is considered to be a simple but effective solution, which may lie in the combination of DrugBank, the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING), and the Database for Annotation, Visualization and Integrated Discovery (DAVID; *Huang, Sherman & Lempicki, 2009*; *Szklarczyk et al., 2015*; *Wishart et al., 2006*). DrugBank is a web-enabled tool that allows researchers to search and mine drug-primary targets information (*Wishart et al., 2008*). Subsequently, STRING database could predict information for primary–secondary targets interaction in silico (*Szklarczyk et al., 2015*).

In this study, we employed DrugBank to widely study flavanone naringenin and obtain related direct protein targets (DPTs) information. The STRING database was then used to explore the interactions between DPTs and indirect protein targets (IPTs), and

protein–protein interaction (PPI) network of naringenin-mediated proteins (including DPTs and IPTs) was subsequently constructed and visualized by Cytoscape based on interaction data obtained in STRING. Next, functions of all naringenin-mediated proteins were investigated through Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis by DAVID. Pivotal protein genes (*ESR1*, *PIK3CA*, *AKT1*, *MAPK1*) were identified among all naringenin-mediated proteins and investigated genomic alterations using cBioPortal database. In general, the results of this functional network analysis help to provide information for integratively understanding mechanisms of naringenin action, and the implications for the prevention and therapy of prostate cancer by naringenin.

MATERIALS AND METHODS

Search for direct protein targets of naringenin

The DrugBank database (https://www.drugbank.ca) is a web-based bioinformatic tool, including numerous drug information such as chemical structure, targets, transporters, biointeractions, and so on (*Wishart et al., 2006*, *2008*). The comprehensive information could be used to facilitate construction of drug-target interactome in silico. Thus, the DrugBank database was employed to search for DPTs of naringenin. The analysis results were then used for further analysis.

Network visualization and KEGG pathway enrichment analysis

Direct protein targets obtained from the DrugBank database were in turn subject to retrieval in the STRING database (http://www.string-db.org/), setting the minimum required interaction score as 0.5 and max number of interactors as 20. The IPTs of naringenin were generated after removing repetitive proteins. Then interactions of all naringenin-mediated proteins were analyzed by the STRING web server, and their PPI network was visualized using Cytoscape software (version 3.6.1) (*Su et al., 2014*). Subsequently, all naringenin-mediated proteins were also carried on functional KEGG pathway enrichment analysis by DAVID with p-value < 0.05.

Exploring cancer genomics data linked to naringenin by cBioPortal

The cBioPortal for Cancer Genomics (http://cbioportal.org) is an open-access platform for cancer researchers to explore multidimensional cancer genomics data (*Cerami et al., 2012*; *Gao et al., 2013*). Biologic insights and clinical applications could be understood using these rich genomic data. In this study, screened protein genes (*ESR1*, *PIK3CA*, *AKT1*, *MAPK1*) from the investigation above were subject to genetic alteration analysis in all prostate cancer studies available in cBioPortal databases. Then, the prostate cancer study with the highest genetic alteration was chosen to analyze the connectivity of the screened protein genes (*ESR1*, *PIK3CA*, *AKT1*, *MAPK1*). *Gao et al.'s (2013)* study helped us to interpret the result of the query in cBioPortal.

RESULTS

Characterization of naringenin DPTs

Some small molecule chemical compounds can directly interact with proteins in organisms, which will result in alteration of the downstream pathway and various

Table 1 Identification of direct protein targets of naringenin using DrugBank.

No.	Uniprot ID	Uniprot name	Gene name
1	P03372	Estrogen receptor	ESR1
2	Q04828	Aldo-keto reductase family 1 member C1	AKR1C1
3	Q16678	Cytochrome P450 1B1	CYP1B1
4	Q9P2N6	KAT8 regulatory NSL complex subunit 3	KANSL3
5	P04278	Sex hormone-binding globulin	SHBG
6	P11511	Aromatase	CYP19A1
7	Q92731	Estrogen receptor beta	ESR2
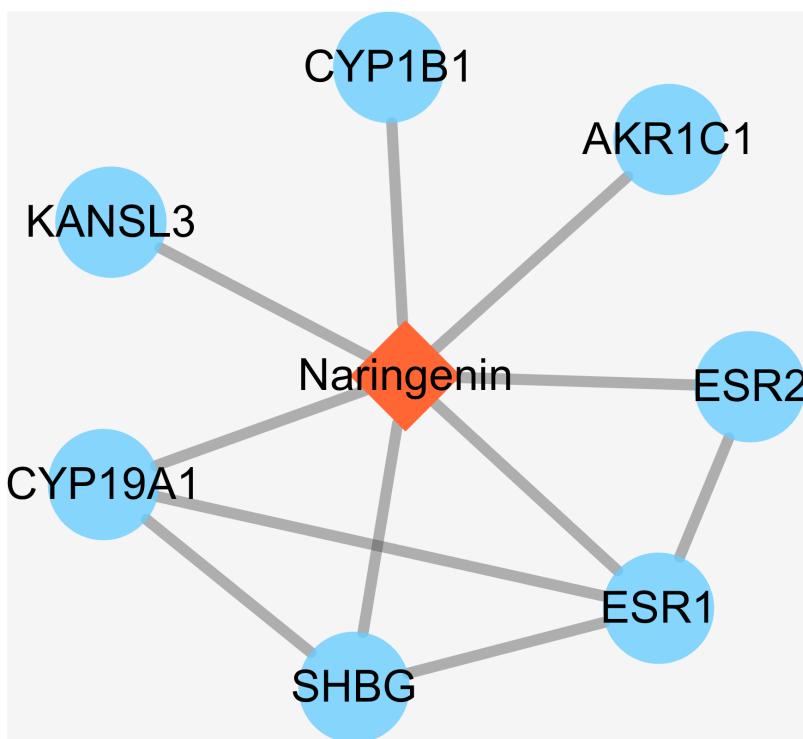
Figure 2 The interactions of naringenin and its DPTs. Full-size ⊡ DOI: 10.7717/peerj.6611/fig-2

physiologic functions. In order to achieve DPTs of naringenin and relevant information, we entered Drugbank using naringenin as input. As a result, the output displayed DB03467 and total of eight primary DPTs. In the category of pharmacological activity, naringenin was classified as antiulcer agents, estrogen antagonists, BCRP/ABCG2 inhibitors, gastrointestinal agents, and hormone antagonists. Subsequent screening demonstrated seven DPTs belonging to human beings, which were presented in Table 1, ESR1, AKR1C1, CYP1B1, KANSL3, SHBG, CYP19A1, and ESR2. In addition, interactions between naringenin and seven DPTs were analyzed and illustrated in Fig. 2. It should be noted three direct target proteins (CYP19A1, SHBG, ESR2) showed a direct association with ESR1, suggesting that ESR1 played a critical role in targeted pathways controlled or mediated by naringenin.
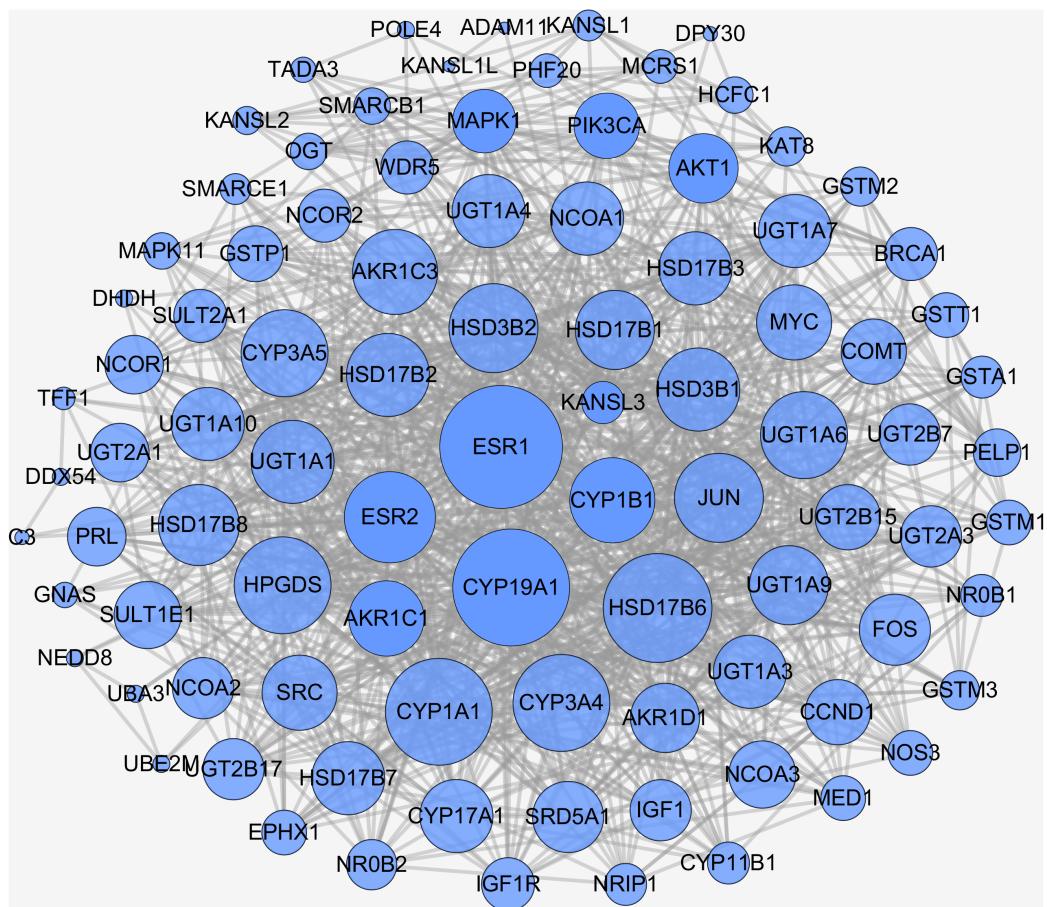
Figure 3 PPI network of naringenin-mediated proteins. The nodes indicate proteins and the edges indicate interaction between proteins. High node degree value was represented by big size and low node degree value was represented by small size. Full-size ⤢ DOI: 10.7717/peerj.6611/fig-3

Visualization and PPI network construction of naringenin-mediated proteins

In biological systems, physiologic functions rely on interactions among diverse proteins, which can be represented by a network consisting of nodes and edges. Thus, there should be of great importance to search for downstream targets of naringenin DPTs. To this end, STRING database was applied to identify DPTs-related proteins and the results were summarized in Table S1. In general, total of 109 naringenin-mediated proteins (Table S2) were generated including seven DPTs and 102 IPTs. Moreover, the interaction data of all naringenin-mediated proteins were obtained using STRING (Table S3) and the PPI network were then visualized by Cytoscape 3.6.1 (Su et al., 2014). As shown in Fig. 3, there were 912 PPI pairs in the network of naringenin-mediated proteins. The nodes indicated proteins and the edges indicated the interactions between these proteins. Next, node degree was evaluated to investigate the centrality of proteins and represented by size, low values to the small size. Ranked by degree value from large to small, top 10 proteins were screened out and showed in Table 2, including three DPTs (ESR1, CYP19A1, ESR2). These findings collectively indicated that biological effects mediated by naringenin are connected to ESR1.

Table 2 The list of top 10 proteins ranked by degree value.

No.	Gene name	Node degree	No.	Gene name	Node degree
1	ESR1	51	6	HPGDS	38
2	CYP19A1	48	7	ESR2	35
3	HSD17B6	44	8	JUN	34
4	CYP1A1	43	9	HSD3B2	34
5	CYP3A4	38	10	UGT1A6	33

KEGG enrichment pathway analysis of naringenin-mediated proteins

As a collection of molecular interaction, reaction, and relation networks, KEGG pathways offer a good understanding of high-level functions and the biological system.
Thus, we performed the KEGG pathway enrichment analysis using DAVID to assess the functional feature of naringenin-mediated proteins (Table S4). As can be seen in Table 3, the top 15 KEGG pathways linked to naringenin-mediated proteins were identified and included steroid hormone biosynthesis, metabolism of xenobiotics by cytochrome P450, chemical carcinogenesis, drug metabolism-cytochrome P450, retinol metabolism, pentose and glucuronate interconversions, ascorbate and aldarate metabolism, ovarian steroidogenesis, drug metabolism-other enzymes, porphyrin and chlorophyl metabolism, prolactin signaling pathway, thyroid hormone signaling pathway, estrogen signaling pathway, metabolic pathways, and prostate cancer. The broad grouping of functional enrichment analysis results indicated that naringenin-mediated proteins were mainly linked to (1) estrogen-related signaling pathways, (2) basal metabolism pathways, (3) cancer-related pathways. Given the importance of ESR1 in all naringenin-mediated proteins, emphasis of biological functions was directed to pathway possessing ESR1. Thus, three ESR1-related pathways were identified: prolactin signaling pathway, thyroid hormone signaling pathway, and estrogen signaling pathway. Besides, because anticarcinogenic properties of naringenin have been reported in diverse malignant tumors as mentioned above, prostate cancer pathway consisting of eight proteins was found: AKT1, MAPK1, IGF1R, AR, CCND1, INS, PIK3CA, IGF1. This result suggested that prostate cancer might be as a phenotype connected to naringenin-mediated proteins.

Genetic alterations connected with naringenin-mediated protein genes in prostate cancer

Previous functional enrichment analysis uncovered the link between naringenin-mediated proteins and prostate cancer pathway. Further exploration was needed to validate this link. Since ESR1 enjoyed the highest centrality among all naringenin-mediated proteins as proved previously, and because three overlapping proteins (PIK3CA, AKT1, MAPK1) associated with ESR1 were found to be connected to prostate cancer pathway as well, the genomic alteration of these four protein genes (*ESR1, PIK3CA, AKT1, MAPK1*) were checked in prostate cancer using cBioPortal. Among 16 prostate cancer studies (*Baca et al., 2013*; *Barbieri et al., 2012*; *Beltran et al., 2016*; *Fraser et al., 2017*;

Table 3 Top 15 enriched KEGG pathways identified using DAVID.

Pathway description	Gene count	Gene	p-value
Steroid hormone biosynthesis	33	CYP3A4, HSD3B2, CYP3A5, HSD3B1, CYP1B1, HSD17B2, HSD17B1, CYP11B1, COMT, UGT1A7, AKR1C3, UGT1A6, UGT1A9, UGT1A3, UGT1A4, UGT2A1, HSD17B6, HSD17B3, SRD5A1, UGT2A3, SULT1E1, HSD17B7, AKR1C1, CYP19A1, HSD17B8, CYP1A1, UGT1A1, UGT1A10, UGT2B17, CYP17A1, UGT2B15, AKR1D1, UGT2B7	4.86E-49
Metabolism of xenobiotics by cytochrome P450	26	GSTA1, CYP3A4, CYP3A5, CYP1B1, SULT2A1, CYP1A1, EPHX1, GSTT1, UGT1A1, DHDH, GSTM1, UGT1A7, UGT1A10, GSTM2, UGT1A6, UGT1A9, UGT2B17, GSTM3, UGT1A3, UGT1A4, UGT2A1, UGT2A3, UGT2B15, UGT2B7, AKR1C1, GSTP1	3.29E-31
Chemical carcinogenesis	24	GSTA1, CYP3A4, CYP3A5, CYP1B1, SULT2A1, CYP1A1, EPHX1, GSTT1, UGT1A1, UGT1A7, GSTM1, UGT1A10, UGT1A6, GSTM2, UGT1A9, UGT2B17, GSTM3, UGT1A3, UGT1A4, UGT2A1, UGT2A3, UGT2B15, UGT2B7, GSTP1	8.08E-27
Drug metabolism-cytochrome P450	20	GSTA1, CYP3A4, CYP3A5, GSTT1, UGT1A1, UGT1A7, GSTM1, UGT1A10, UGT1A6, GSTM2, UGT1A9, UGT2B17, GSTM3, UGT1A3, UGT1A4, UGT2A1, UGT2A3, UGT2B15, GSTP1, UGT2B7	6.4E-22
Retinol metabolism	16	CYP3A4, CYP3A5, CYP1A1, UGT1A1, UGT1A7, UGT1A6, UGT1A10, UGT1A9, UGT2B17, UGT1A3, UGT1A4, UGT2A1, HSD17B6, UGT2A3, UGT2B15, UGT2B7	3.57E-16
Pentose and glucuronate interconversions	13	UGT1A7, UGT1A10, UGT1A6, UGT1A9, UGT2B17, UGT1A3, UGT1A4, UGT2A1, UGT2A3, UGT2B15, UGT1A1, DHDH, UGT2B7	1.02E-15
Ascorbate and aldarate metabolism	12	UGT1A7, UGT1A10, UGT1A6, UGT1A9, UGT2B17, UGT1A3, UGT1A4, UGT2A1, UGT2A3, UGT2B15, UGT1A1, UGT2B7	3.81E-15
Ovarian steroidogenesis	14	AKR1C3, HSD3B2, IGF1R, CYP17A1, HSD3B1, CYP1B1, CYP1A1, HSD17B2, INS, HSD17B1, IGF1, GNAS, HSD17B7, CYP19A1	6.86E-15
Drug metabolism-other enzymes	13	CYP3A4, UGT1A7, UGT1A10, UGT1A6, UGT1A9, UGT2B17, UGT1A3, UGT1A4, UGT2A1, UGT2A3, UGT2B15, UGT1A1, UGT2B7	1.01E-13
Porphyrin and chlorophyl metabolism	12	UGT1A7, UGT1A10, UGT1A6, UGT1A9, UGT2B17, UGT1A3, UGT1A4, UGT2A1, UGT2A3, UGT2B15, UGT1A1, UGT2B7	1.10E-12
Prolactin signaling pathway	12	AKT1, MAPK1, FOS, CYP17A1, CCND1, INS, ESR1, PIK3CA, MAPK11, ESR2, PRL, SRC	4.99E-10
Thyroid hormone signaling pathway	12	AKT1, MAPK1, NCOA1, CCND1, NCOA2, NCOA3, ESR1, PIK3CA, NCOR1, MYC, SRC, MED1	9.09E-08
Estrogen signaling pathway	10	AKT1, MAPK1, FOS, JUN, ESR1, PIK3CA, NOS3, GNAS, ESR2, SRC	2.26E-06
Metabolic pathways	32	CYP3A4, HSD3B2, CYP3A5, HSD3B1, HSD17B2, HSD17B1, CYP11B1, COMT, AKR1C3, UGT1A7, UGT1A6, UGT1A9, POLE4, UGT1A3, UGT1A4, UGT2A1, HSD17B6, HSD17B3, NOS3, UGT2A3, HPGDS, HSD17B7, CYP19A1, HSD17B8, CYP1A1, UGT1A1, UGT1A10, UGT2B17, CYP17A1, UGT2B15, AKR1D1, UGT2B7	1.18E-05
Prostate cancer	8	AKT1, MAPK1, IGF1R, AR, CCND1, INS, PIK3CA, IGF1	7.70E-05

Gao et al., 2014; *Grasso et al., 2012*; *Hieronymus et al., 2014*; *Knudsen & Scher, 2009*; *Cancer Genome Atlas Research Network, 2015*; *Robinson et al., 2015*; *Taylor et al., 2010*), gene alterations ranged from 0% to 31.58% as displayed in Fig. 4. Because of the most pronounced genetic alterations among all prostate cancer studies available in cBioPortal, the NEPC study (*Beltran et al., 2016*) was selected individually to view relevant genomic changes of four genes. A concise and compact summary of alterations in three queried genes
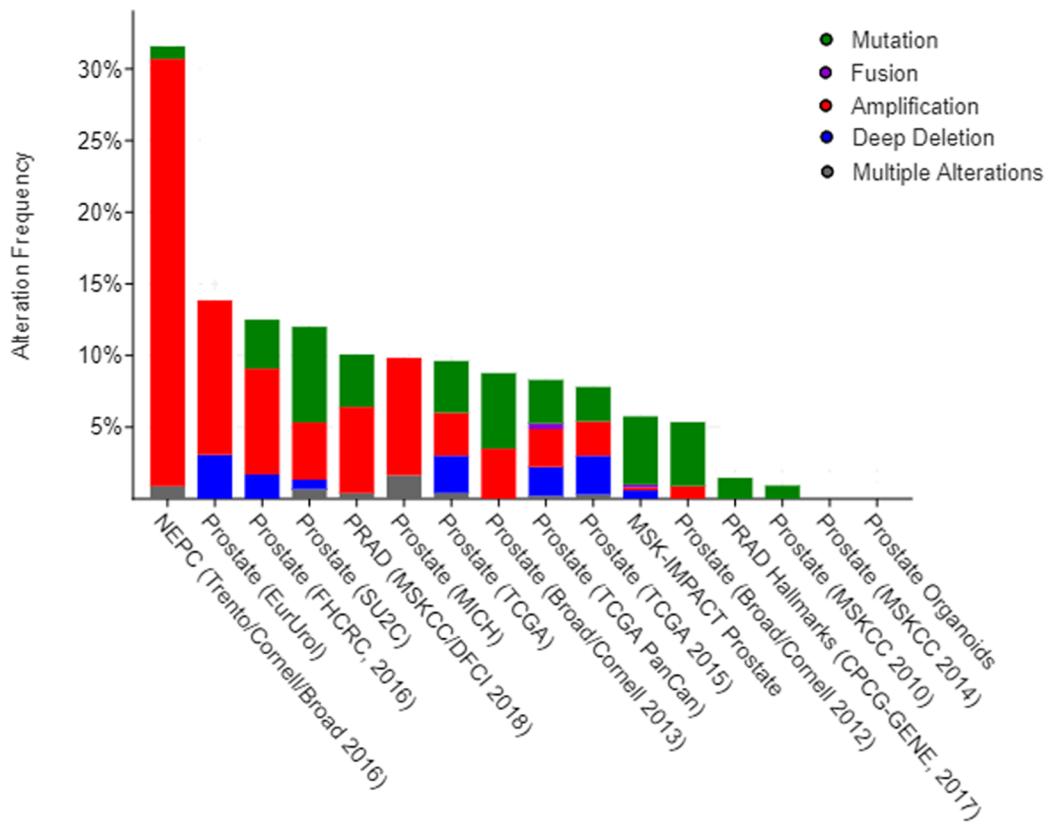
Figure 4 Overview of changes on *ESR1*, *PIK3CA*, *AKT1*, and *MAPK1* genes in genomics data sets available in 16 different prostate cancer studies. Full-size ◩ DOI: 10.7717/peerj.6611/fig-4

(*ESR1*, *PIK3CA*, *AKT1*, *MAPK1*) was shown in Fig. 5 using OncoPrint. The results presented that most gene alterations belonged to amplification. Additional mutual exclusivity analysis indicated that every gene pair exhibited significant (*p*-value < 0.05) co-occurrence in prostate samples in the study of NEPC (Table 4). The co-occurrence of *ESR1* and other three genes revealed a central axis function for *ESR1* under naringenin control.

Subsequently, the Network tab embedded in cBioportal was exploited to explore the interactive relationship between four selected genes and genes that were altered in NEPC prostate cancer study. A query of *ESR1*, *PIK3CA*, *AKT1*, and *MAPK1* automatically generated a network containing 50 neighbor genes of four query genes, and legends were available to explain network symbols (Fig. 6A). To manage network complexity, we filtered neighbors by 45% alteration, such that only *AR* gene with the highest alteration frequency remained in addition to four query genes (Fig. 6B). The pruned network revealed the potential interactions between naringenin-mediated genes and altered genes in prostate cancer samples. Moreover, specific cancer drugs acting on ESR1, PIK3CA, AKT1, and MAPK1 were displayed in Fig. 5. ESR1 was the main target of most FDA approved drugs (represented by yellow hexagon) in the network, providing a molecular basis for potential clinical applications of naringenin to treat prostate cancer targeting ESR1.
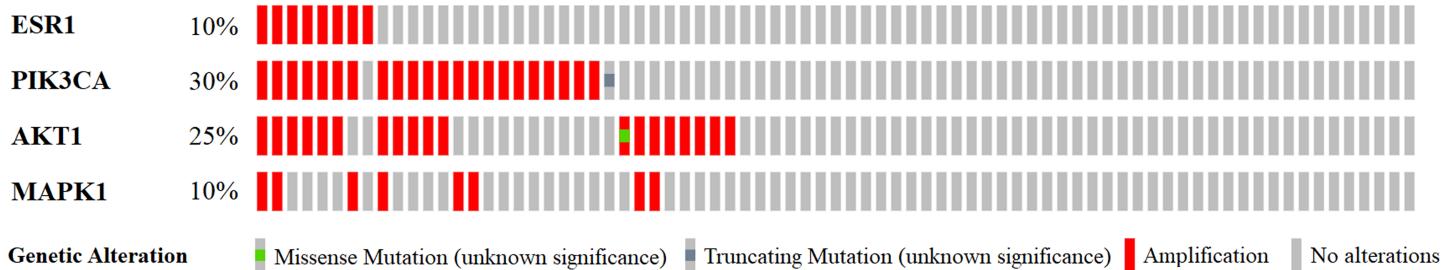
Figure 5 A visual summary of alteration across a set of prostate samples (data taken from the NEPC studies, Nat Med 2016) (*Beltran et al., 2016*) based on a query of four genes *ESR1*, *PIK3CA*, *AKT1*, and *MAPK1*. Different genomic alterations are summarized and color coded presented by % changes in particular affected genes in individual tumor samples. Each row represents a gene, and each column represents a tumor sample.
Full-size ☑ DOI: 10.7717/peerj.6611/fig-5

Table 4 Mutual exclusivity analysis of four selected genes (*ESR1*, *AKT1*, *PIK3CA*, *MAPK1*) in NEPC study.

Gene A	Gene B	*p*-value	Log2 odds ratio	Association
ESR1	AKT1	<0.001	>3	Tendency toward co-occurrence
ESR1	PIK3CA	<0.001	>3	Tendency toward co-occurrence
ESR1	MAPK1	0.003	>3	Tendency toward co-occurrence
PIK3CA	AKT1	<0.001	2.733	Tendency toward co-occurrence
AKT1	MAPK1	0.002	>3	Tendency toward co-occurrence
PIK3CA	MAPK1	0.005	>3	Tendency toward co-occurrence

DISCUSSION

Since naringenin was reported by *Hendershott & Walker (1959)*, there has been an increasing number of publications on naringenin and more than 2,400 publications have accumulated on PubMed to date. A wide range of biological and cellular activities have been reported for naringenin, including relevant targets and biological pathways. These findings suggested that naringenin connected with a plethora of diseases and possessed a fascinating nature. However, there are still barriers to systematically understand how naringenin facilitates its wide range of beneficial effects. As such, a bridge between naringenin and its direct and indirect targets needs to be established. To this end, functional network analysis (*Hsieh et al., 2016*; *Shi et al., 2017*) with new analytical ways or platforms is introduced in this study and enables to explore naringenin-mediated proteins, thereby elucidating connectivity between protein genes and phenotype.

In our study, functional network analysis was performed by using a series of web-based bioinformatic tools. Firstly, the feasibility of analysis for connectivity between naringenin targets and its phenotypes was demonstrated by using DrugBank and STRING, resulting in the identification of seven DPTs (ESR1, AKR1C1, CYP1B1, KANSL3, SHBG, CYP19A1, ESR2), 102 IPTs (Table S2). Secondly, the KEGG enrichment analysis conducted by DAVID identified three ESR1-related signaling pathways and prostate cancer pathway, which were significantly altered by naringenin-mediated proteins. As supporting evidence, previous studies have observed that naringenin exhibits
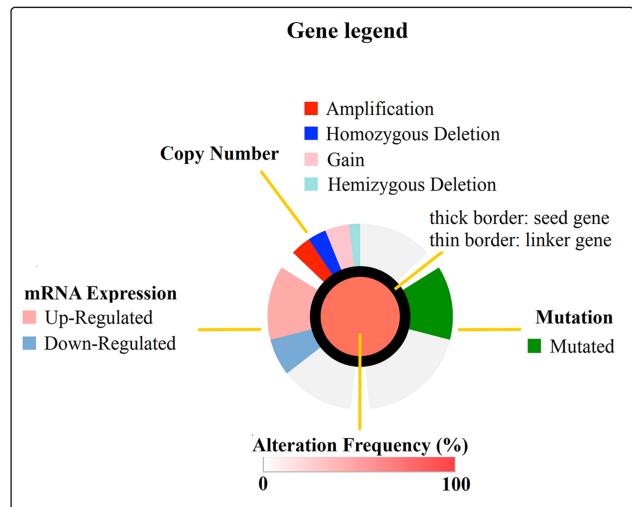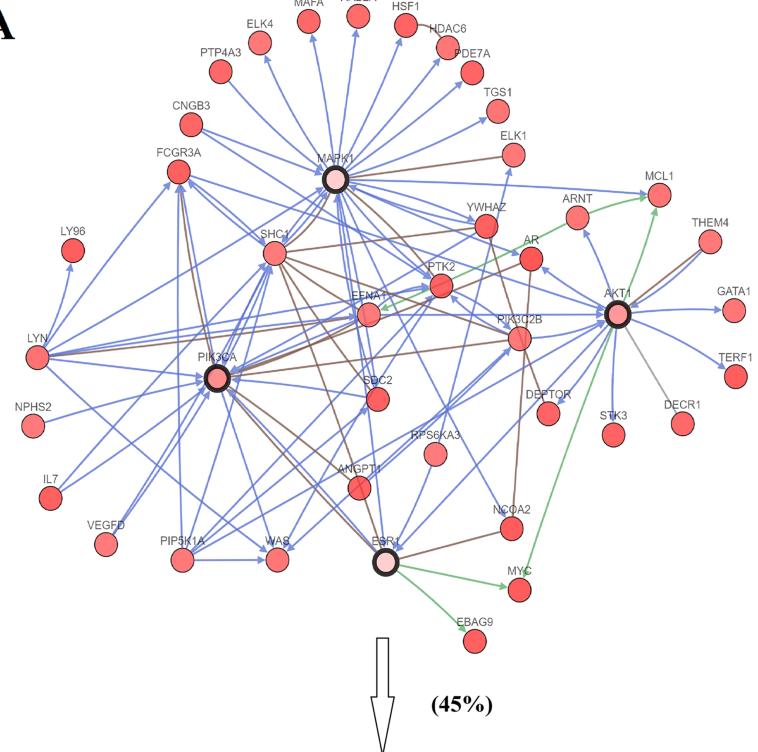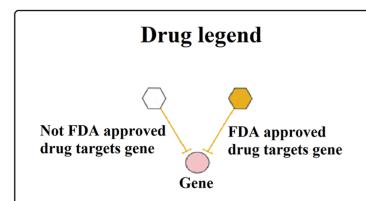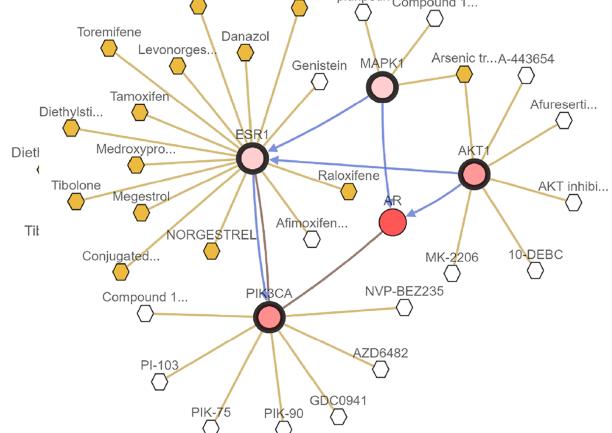
Figure 6 A visual display of the gene network connected to ESR1/PIK3CA/AKT1/MAPK1 in prostate adenocarcinoma (based on the NEPC study, Nat Med 2016) (*Beltran et al., 2016*). (A) Cross-cancer alteration summary for ESR1/PIK3CA/AKT1/MAPK1 mined from the cBioPortal for Cancer Genomics. Multidimensional genomic details are shown for seed genes *ESR1*, *PIK3CA*, *AKT1*, and *MAPK1*. A darker shade of red indicates increased frequency of alteration in prostate cancer. (B) Neighboring genes connected to the four query genes, filtered by alterations (45%).

Full-size ☑ DOI: 10.7717/peerj.6611/fig-6

antineoplastic property against most solid tumors including breast and colorectal (*Abaza et al., 2015*), bladder (*Liao et al., 2014*), prostate (*Lim et al., 2017*), and so on. Thus, the association between prostate cancer and the beneficial effects exerted by naringenin in cancer was then explored and assessed by the genetic alternations in four protein genes (*ESR1*, *PIK3CA*, *AKT1*, and *MAPK1*) which were revealed by naringenin

associated three ESR1-related signaling pathways and prostate cancer pathway. Most of the genetic alterations in *ESR1*, *PIK3CA*, *AKT1*, and *MAPK1* were amplifications, suggesting an excess expression in prostate cancer. Consistent with our study, *Fu et al. (2014)* pointed out it that the genetic polymorphisms in *ESR1* gene could cause transcription change, resulting in the influence the risk of prostate cancer. PI3K/AKT pathway including PIK3CA and AKT1 is a well-known pathway involved in the regulation of cell proliferation, metastasis, and apoptosis (*Mayer & Arteaga, 2016*). Recent research even found that *PIK3CA* amplification had a correlation with poor survival of patients with prostatic carcinoma (*Pearson et al., 2018*). To the present, *MAPK1* is a famous oncogene, acting as a signal transduction node of various upstream signals such as proliferation, inhibition of apoptosis, and so on (*Chen et al., 2015*). Moreover, the mutual exclusivity analysis discovered a tendency toward co-occurrence between *ESR1* and three overlapping genes. Hence, the results were in good agreement with it that *ESR1* acted as a major driver of anti-carcinogenic efficiency in prostate cancer. In addition, *PIK3CA*, *AKT1*, and *MAPK1* directly interacted with *AR* (Fig. 6B), which was a naringenin-mediated protein gene as well and participated in prostate cancer pathway. As support, AR has been reported to be crucial to prostate cell growth and development (*Heinlein & Chang, 2002*). Therefore, a hypothesis is currently proposed that naringenin acts through a series of genes (*ESR1*, *PIK3CA*, *AKT1*, *MAPK1*, and *AR*) to control prostate cancer cell proliferation.

Prostate cancer is the most common malignancy in male cancer patients with high mortality, accounting for 23% of all new cancer cases worldwide (*Siegel, Miller & Jemal, 2018*). The gene-phenotype connectivity liaised by naringenin revealed that the regulation of ESR1 by naringenin might be a major driver for the chemoprotective salutary effects on prostate adenocarcinoma. The candidate genes identified may facilitate the comprehension of genomic results and be considered to provide information useful for guiding further research of naringenin and future drug development. However, some challenges of this analysis remain to be investigated and solved: (1) whether the connectivity existing between naringenin and prostate cancer can extend to other malignant tumors is still essential to explore; (2) the role of genes under naringenin control detected in this research must be verified in prostate cancer.

CONCLUSIONS

In conclusion, functional network analysis based on a series of online tools including DrugBank, STRING, DAVID, and cBioPortal has been applied to mine and integrate knowledge of naringenin biological action. The retrieval of publicly available computational databases unraveled the connectivity existing between naringenin and prostate cancer. A hypothesis currently being considered is that naringenin could regulate prostate cancer via ESR1 signaling axis. Naringenin might be promising as an alternative chemotherapy or chemoprevention for prostate cancer. Overall, this functional network analysis provided a reasonable hypothesis and assisted in the acceleration of naringenin biology research.

ADDITIONAL INFORMATION AND DECLARATIONS

REFERENCES

Abaza MSI, Orabi K, Al-Quattan E, Al-Attiyah R. 2015. Growth inhibitory and chemo-sensitization effects of naringenin, a natural flavanone purified from *Thymus vulgaris*, on human breast and colorectal cancer. *Cancer Cell International* **15(1)**:46 DOI 10.1186/s12935-015-0194-0.

Baca SC, Prandi D, Lawrence MS, Mosquera JM, Romanel A, Drier Y, Park K, Kitabayashi N, MacDonald TY, Ghandi M, Van Allen E, Kryukov GV, Sboner A, Theurillat J-P, Soong TD, Nickerson E, Auclair D, Tewari A, Beltran H, Onofrio RC, Boysen G, Guiducci C, Barbieri CE, Cibulskis K, Sivachenko A, Carter SL, Saksena G, Voet D, Ramos AH, Winckler W, Cipicchio M, Ardlie K, Kantoff PW, Berger MF, Gabriel SB, Golub TR, Meyerson M, Lander ES, Elemento O, Getz G, Demichelis F, Rubin MA, Garraway LA. 2013. Punctuated evolution of prostate cancer genomes. *Cell* **153(3)**:666–677 DOI 10.1016/j.cell.2013.03.021.

Barbieri CE, Baca SC, Lawrence MS, Demichelis F, Blattner M, Theurillat J-P, White TA, Stojanov P, Van Allen E, Stransky N, Nickerson E, Chae S-S, Boysen G, Auclair D, Onofrio RC, Park K, Kitabayashi N, MacDonald TY, Sheikh K, Vuong T, Guiducci C, Cibulskis K, Sivachenko A, Carter SL, Saksena G, Voet D, Hussain WM, Ramos AH,

Winckler W, Redman MC, Ardlie K, Tewari AK, Mosquera JM, Rupp N, Wild PJ, Moch H, Morrissey C, Nelson PS, Kantoff PW, Gabriel SB, Golub TR, Meyerson M, Lander ES, Getz G, Rubin MA, Garraway LA. 2012. Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. *Nature Genetics* **44(6)**:685–689 DOI 10.1038/ng.2279.

Beltran H, Prandi D, Mosquera JM, Benelli M, Puca L, Cyrta J, Marotz C, Giannopoulou E, Varambally S, Tomlins SA, Nanus DM, Tagawa ST, Van Allen EM, Elemento O, Sboner A, Garraway LA, Rubin MA, Demichelis F. 2016. Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. *Nature Medicine* **22(3)**:298–305 DOI 10.1038/nm.4045.

Cancer Genome Atlas Research Network. 2015. The molecular taxonomy of primary prostate cancer. *Cell* **163(4)**:1011–1025 DOI 10.1016/j.cell.2015.10.025.

Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy Bülent A, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, Antipin Y, Reva B, Goldberg AP, Sander C, Schultz N. 2012. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discovery* **2(5)**:401–404 DOI 10.1158/2159-8290.CD-12-0095.

Chen QG, Zhou W, Han T, Du SQ, Li ZH, Zhang Z, Shan GY, Kong CZ. 2015. MiR-378 suppresses prostate cancer cell growth through downregulation of MAPK1 in vitro and in vivo. *Tumor Biology* **37(2)**:2095–2103 DOI 10.1007/s13277-015-3996-8.

Fraser M, Sabelnykova VY, Yamaguchi TN, Heisler LE, Livingstone J, Huang V, Shiah Y-J, Yousif F, Lin X, Masella AP, Fox NS, Xie M, Prokopec SD, Berlin A, Lalonde E, Ahmed M, Trudel D, Luo X, Beck TA, Meng A, Zhang J, D'Costa A, Denroche RE, Kong H, Espiritu SMG, Chua MLK, Wong A, Chong T, Sam M, Johns J, Timms L, Buchner NB, Orain Mèle, Picard Vérie, Hovington Hène, Murison A, Kron K, Harding NJ, P'ng C, Houlahan KE, Chu KC, Lo B, Nguyen F, Li CH, Sun RX, De Borja R, Cooper CI, Hopkins JF, Govind SK, Fung C, Waggott D, Green J, Haider S, Chan-Seng-Yue MA, Jung E, Wang Z, Bergeron A, Dal Pra A, Lacombe L, Collins CC, Sahinalp C, Lupien M, Fleshner NE, He HH, Fradet Y, Tetu B, Van Der Kwast T, McPherson JD, Bristow RG, Boutros PC. 2017. Genomic hallmarks of localized, non-indolent prostate cancer. *Nature* **541(7637)**:359–364 DOI 10.1038/nature20788.

Fu C, Dong W-Q, Wang A, Qiu G. 2014. The influence of ESR1 rs9340799 and ESR2 rs1256049 polymorphisms on prostate cancer risk. *Tumor Biology* **35(8)**:8319–8328 DOI 10.1007/s13277-014-2086-7.

Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, Cerami E, Sander C, Schultz N. 2013. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Science signaling* **6(269)**:pl1 DOI 10.1126/scisignal.2004088.

Gao D, Vela I, Sboner A, Iaquinta PJ, Karthaus WR, Gopalan A, Dowling C, Wanjala JN, Undvall EA, Arora VK, Wongvipat J, Kossai M, Ramazanoglu S, Barboza LP, Di W, Cao Z, Zhang QF, Sirota I, Ran L, MacDonald TY, Beltran H, Mosquera J-M, Touijer KA, Scardino PT, Laudone VP, Curtis KR, Rathkopf DE, Morris MJ, Danila DC, Slovin SF, Solomon SB, Eastham JA, Chi P, Carver B, Rubin MA, Scher HI, Clevers H, Sawyers CL, Chen Y. 2014. Organoid cultures derived from patients with advanced prostate cancer. *Cell* **159(1)**:176–187 DOI 10.1016/j.cell.2014.08.016.

Grasso CS, Wu Y-M, Robinson DR, Cao X, Dhanasekaran SM, Khan AP, Quist MJ, Jing X, Lonigro RJ, Brenner JC, Asangani IA, Ateeq B, Chun SY, Siddiqui J, Sam L, Anstett M, Mehra R, Prensner JR, Palanisamy N, Ryslik GA, Vandin F, Raphael BJ, Kunju LP, Rhodes DR, Pienta KJ, Chinnaiyan AM, Tomlins SA. 2012. The mutational landscape of

lethal castration-resistant prostate cancer. *Nature* **487(7406)**:239–243
DOI 10.1038/nature11125.

Gumushan Aktas H, Akgun T. 2018. Naringenin inhibits prostate cancer metastasis by
blocking voltage-gated sodium channels. *Biomedicine & Pharmacotherapy* **106**:770–775
DOI 10.1016/j.biopha.2018.07.008.

Heinlein CA, Chang C. 2002. The roles of androgen receptors and androgen-binding proteins
in nongenomic androgen actions. *Molecular Endocrinology* **16(10)**:2181–2187
DOI 10.1210/me.2002-0070.

Hendershott CH, Walker DR. 1959. Identification of a growth inhibitor from extracts of dormant
peach flower buds. *Science* **130(3378)**:798–800 DOI 10.1126/science.130.3378.798-a.

Hernández-Aquino E, Muriel P. 2018. Beneficial effects of naringenin in liver diseases:
molecular mechanisms. *World Journal of Gastroenterology* **24(16)**:1679–1707
DOI 10.3748/wjg.v24.i16.1679.

**Hieronymus H, Schultz N, Gopalan A, Carver BS, Chang MT, Xiao Y, Heguy A, Huberman K,
Bernstein M, Assel M, Murali R, Vickers A, Scardino PT, Sander C, Reuter V, Taylor BS,
Sawyers CL. 2014.** Copy number alteration burden predicts prostate cancer relapse.
Proceedings of the National Academy of Sciences of the United States of America
111(30):11139–11144 DOI 10.1073/pnas.1411446111.

Hsieh TC, Wu ST, Bennett DJ, Doonan BB, Wu E, Wu JM. 2016. Functional/activity network
(FAN) analysis of gene-phenotype connectivity liaised by grape polyphenol resveratrol.
Oncotarget **7(25)**:38670–38680 DOI 10.18632/oncotarget.9578.

Huang DW, Sherman BT, Lempicki RA. 2009. Systematic and integrative analysis of large
gene lists using DAVID bioinformatics resources. *Nature Protocols* **4(1)**:44–57
DOI 10.1038/nprot.2008.211.

Knudsen KE, Scher HI. 2009. Starving the addiction: new opportunities for durable
suppression of AR signaling in prostate cancer. *Clinical Cancer Research* **15(15)**:4792–4798
DOI 10.1158/1078-0432.CCR-08-2660.

Lan K, Wang D-T, Fong S, Liu L-S, Wong KKL, Dey N. 2018. A survey of data mining and deep
learning in bioinformatics. *Journal of Medical Systems* **42(8)**:139 DOI 10.1007/s10916-018-1003-9.

Liao AC, Kuo CC, Huang YC, Yeh CW, Hseu YC, Liu JY, Hsu LS. 2014. Naringenin
inhibits migration of bladder cancer cells through downregulation of AKT and MMP-2.
Molecular Medicine Reports **10(3)**:1531–1536 DOI 10.3892/mmr.2014.2375.

Lim W, Park S, Bazer FW, Song G. 2017. Naringenin-induced apoptotic cell death in prostate
cancer cells is mediated via the PI3K/AKT and MAPK signaling pathways. *Journal of Cellular
Biochemistry* **118(5)**:1118–1131 DOI 10.1002/jcb.25729.

**Maatouk M, Elgueder D, Mustapha N, Chaaban H, Bzéouich IM, Loannou I, Kilani S, Ghoul M,
Ghedira K, Chekir-Ghedira L. 2016.** Effect of heated naringenin on immunomodulatory
properties and cellular antioxidant activity. *Cell Stress and Chaperones* **21(6)**:1101–1109
DOI 10.1007/s12192-016-0734-0.

Mayer IA, Arteaga CL. 2016. The PI3K/AKT pathway as a target for cancer treatment.
Annual Review of Medicine **67(1)**:11–28 DOI 10.1146/annurev-med-062913-051343.

Park JH, Jin C-Y, Lee BK, Kim G-Y, Choi YH, Jeong YK. 2008. Naringenin induces apoptosis
through downregulation of Akt and caspase-3 activation in human leukemia THP-1 cells.
Food and Chemical Toxicology **46(12)**:3684–3690 DOI 10.1016/j.fct.2008.09.056.

Patel K, Singh GK, Patel DK. 2018. A review on pharmacological and analytical aspects of
naringenin. *Chinese Journal of Integrative Medicine* **24(7)**:551–560
DOI 10.1007/s11655-014-1960-x.

Pearson HB, Li J, Meniel VS, Fennell CM, Waring P, Montgomery KG, Rebello RJ, Macpherson AA, Koushyar S, Furic L, Cullinane C, Clarkson RW, Smalley MJ, Simpson KJ, Phesse TJ, Shepherd PR, Humbert PO, Sansom OJ, Phillips WA. 2018. Identification of Pik3ca mutation as a genetic driver of prostate cancer that cooperates with Pten loss to accelerate progression and castration-resistant growth. *Cancer Discovery* **8(6)**:764–779 DOI 10.1158/2159-8290.CD-17-0867.

Robinson D, Van Allen EM, Wu Y-M, Schultz N, Lonigro RJ, Mosquera J-M, Montgomery B, Taplin M-E, Pritchard CC, Attard G, Beltran H, Abida W, Bradley RK, Vinson J, Cao X, Vats P, Kunju LP, Hussain M, Feng FY, Tomlins SA, Cooney KA, Smith DC, Brennan C, Siddiqui J, Mehra R, Chen Y, Rathkopf DE, Morris MJ, Solomon SB, Durack JC, Reuter VE, Gopalan A, Gao J, Loda M, Lis RT, Bowden M, Balk SP, Gaviola G, Sougnez C, Gupta M, Yu EY, Mostaghel EA, Cheng HH, Mulcahy H, True LD, Plymate SR, Dvinge H, Ferraldeschi R, Flohr P, Miranda S, Zafeiriou Z, Tunariu N, Mateo J, Perez-Lopez R, Demichelis F, Robinson BD, Schiffman M, Nanus DM, Tagawa ST, Sigaras A, Eng KW, Elemento O, Sboner A, Heath EI, Scher HI, Pienta KJ, Kantoff P, De Bono JS, Rubin MA, Nelson PS, Garraway LA, Sawyers CL, Chinnaiyan AM. 2015. Integrative clinical genomics of advanced prostate cancer. *Cell* **161(5)**:1215–1228 DOI 10.1016/j.cell.2015.05.001.

Sabarinathan D, Mahalakshmi P, Vanisree AJ. 2011. Naringenin, a flavanone inhibits the proliferation of cerebrally implanted C6 glioma cells in rats. *Chemico-Biological Interactions* **189(1–2)**:26–36 DOI 10.1016/j.cbi.2010.09.028.

Shi J, Dong B, Zhou P, Guan W, Peng Y. 2017. Functional network analysis of gene-phenotype connectivity associated with temozolomide. *Oncotarget* **8(50)**:87554–87567 DOI 10.18632/oncotarget.20848.

Siegel RL, Miller KD, Jemal A. 2018. Cancer statistics, 2018. *CA Cancer Journal for Clinicians* **68(1)**:7–30 DOI 10.3322/caac.21442.

Su G, Morris JH, Demchak B, Bader GD. 2014. Biological network exploration with cytoscape 3. *Current Protocols in Bioinformatics* **47(1)**:8.13.1–8.13.24 DOI 10.1002/0471250953.bi0813s47.

Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, Simonovic M, Roth A, Santos A, Tsafou KP, Kuhn M, Bork P, Jensen LJ, Von Mering C. 2015. STRING v10: protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Research* **43(D1)**:D447–D452 DOI 10.1093/nar/gku1003.

Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, Arora VK, Kaushik P, Cerami E, Reva B, Antipin Y, Mitsiades N, Landers T, Dolgalev I, Major JE, Wilson M, Socci ND, Lash AE, Heguy A, Eastham JA, Scher HI, Reuter VE, Scardino PT, Sander C, Sawyers CL, Gerald WL. 2010. Integrative genomic profiling of human prostate cancer. *Cancer Cell* **18(1)**:11–22 DOI 10.1016/j.ccr.2010.05.026.

Wishart DS, Knox C, Guo AC, Cheng D, Shrivastava S, Tzur D, Gautam B, Hassanali M. 2008. DrugBank: a knowledgebase for drugs, drug actions and drug targets. *Nucleic Acids Research* **36(suppl_1)**:D901–D906 DOI 10.1093/nar/gkm958.

Wishart DS, Knox C, Guo AC, Shrivastava S, Hassanali M, Stothard P, Chang Z, Woolsey J. 2006. DrugBank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Research* **34(90001)**:D668–D672 DOI 10.1093/nar/gkj067.

Yen H-R, Liu C-J, Yeh C-C. 2015. Naringenin suppresses TPA-induced tumor invasion by suppressing multiple signal transduction pathways in human hepatocellular carcinoma cells. *Chemico-Biological Interactions* **235**:1–9 DOI 10.1016/j.cbi.2015.04.003.

Zaidun NH, Thent ZC, Latiff AA. 2018. Combating oxidative stress disorders with citrus flavonoid: naringenin. *Life Sciences* **208**:111–122 DOI 10.1016/j.lfs.2018.07.017.